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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C11D 3/386 A1

(11) International Publication Number: WO 98/13462

(43) International Publication Date: 2 April 1998 (02.04.98)

(21) International Application Number: PCT/US97/16626

(22) International Filing Date: 19 September 1997 (19.09.97)

(30) Priority Data:
60/026,647 24 September 1996 (24.09.96) US

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(81) Designated States: BR, CA, CN, JP, MX, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LIQUID DETERGENTS CONTAINING PROTEOLYTIC ENZYME, PEPTIDE ALDEHYDE AND A SOURCE OF BORIC ACID

(57) Abstract

Aqueous liquid detergent compositions are described which comprise a proteolytic enzyme wherein the proteolytic activity is reversibly inhibited by a peptide aldehyde and a source of boric acid. In a particularly preferred embodiment, liquid detergent compositions are disclosed which contain a detersive surfactant, a proteolytic enzyme, a peptide aldehyde, boric acid, and diol ions. The combination of peptide aldehyde and boric acid act to provide improved stability of protease and other enzymes.

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LIQUID DETERGENTS CONTAINING PROTEOLYTIC ENZYME, PEPTIDE ALDEHYDE AND A SOURCE OF BORIC ACID

TECHNICAL FIELD

This invention relates to liquid detergent compositions containing enzymes. More specifically, this invention pertains to liquid detergent compositions containing a detersive surfactant, a proteolytic enzyme, a peptide aldehyde, and boric acid. The combination of peptide aldehyde and boric acid act to provide improved protease inhibitor benefits.

BACKGROUND OF THE INVENTION

Protease-containing liquid aqueous detergents are well-known, especially in the context of laundry washing and care. A commonly encountered problem in such protease-containing liquid aqueous detergents is the degradation phenomenon by the proteolytic enzyme of second enzymes in the composition, such as lipase, amylase and cellulase, or on the protease itself. As a result, the stability of the second enzyme or the protease itself in the detergent composition is affected and the detergent composition consequently performs less well.

In response to this problem, it has been proposed to use various protease inhibitors or stabilizers. For instance various references have proposed the use of the following compounds to aid in the stabilization of enzymes: benzamidine hydrochloride, lower aliphatic alcohols or carboxylic acids, mixtures of a polyol and a boron compound, aromatic borate esters, and calcium, particularly calcium formate. Recently, it was discovered that certain peptide aldehydes act to stabilize protease enzyme.

Although these compounds have been used to varying success in liquid detergents, they are not free of problems. For example peptide aldehydes are rather expensive and create complexities for the formulators, especially for liquid detergents. Other inhibitors such as calcium and boric acids are less expensive but do not stabilize enzymes as well as peptide aldehydes. It is thus an object of the present invention to provide a protease inhibitor system which is economical, effective and suitable for use in a liquid detergent composition.

In response to this object, the present invention proposes to use a combination of boric acid or a source thereof and peptide aldehydes as reversible protease

inhibitors in aqueous liquid detergent compositions. The presence of both boric acid and peptide aldehyde provides economical, improved stabilization of the protease. This novel combination provides the formulator added flexibility in designing a stabilization system. The levels of peptide aldehyde and boric acid can be adjusted to deliver the most cost effective formula and to minimize product stability problems that often arise from the presence of divalent ions in a liquid detergent matrix.

In particular, the present invention allows for the use of very low levels of peptide aldehydes in the liquid detergent compositions herein. This is particularly critical in the formulation of relatively inexpensive, concentrated liquid detergent compositions which are encompassed by the present invention.

Because the combination of boric acid and peptide aldehydes are so efficient in inhibiting proteases, another advantage of the present invention is that even enzymes which are highly sensitive to proteolytic degradation can now be incorporated in liquid detergent compositions comprising a protease.

BACKGROUND ART

It has been proposed to use various protease inhibitors or stabilizers. For instance, US 4,566,985 proposes to use benzamidine hydrochloride; EP 376 705 proposes to use lower aliphatic alcohols or carboxylic acids; EP 381 262 proposes to use a mixture of a polyol and a boron compound; and EP91870072.5 proposes to use aromatic borate esters. See also U.S. Pat. No. 5,030,378 issued July 9, 1991. Also see US4,261,868; US4,404,115; US4,318,818; and EP130,756.

The use of peptide derivatives for the inhibition of proteins appears to have been disclosed in therapeutic applications. EP 293 881 discloses the use of peptide boronic acids as inhibitors of trypsin-like serine proteases. EP 185 390 and US 4,399,065 disclose the use of certain peptide aldehydes derivatives for the inhibition of blood coagulation. J 90029670 discloses the use of optically active alpha amino aldehydes for the inhibition of enzymes in general. See also "Inhibition of Thrombin and Trypsin by Tripeptide Aldehydes", Int. J. Peptide Protein Res., Vol 12 (1978), pp. 217-221; Gaal, Bacsy & Rappay, and "Tripeptide Aldehyde Protease Inhibitors May Depress in Vitro Prolactin and Growth Hormone Release" Endocrinology, Vol. 116, No. 4 (1985), pp. 1426-1432; Rappay, Makara, Bajusz & Nagy. Certain peptide aldehydes have also been disclosed in EP-A-473 502 for inhibiting protease-mediated skin irritation.

In particular see EP185,390, WO94/04651, published 3 March 1994, WO94/04652, published 3 March 1994, EP 583,536, published February 23, 1994, EP 583,535, published February 3, 1994, EP 583,534, published February 23, 1994,

WO 93/13125, published July 8, 1993, US4,529,525, US4,537,706, US4,537,707, and US5,527,487.

SUMMARY OF THE INVENTION

The invention herein is a liquid detergent composition comprising:

a) an effective amount of a detersive surfactant;

b) an active proteolytic enzyme;

c) a boron composition comprising a source of boric acid and a diol; and

d) a peptide aldehyde having the formula:

Z-B-NH-CH(R)-C(O)H

wherein B is a peptide chain comprising from 1 to 5 amino acid moieties; Z is an N-capping moiety selected from the group consisting of phosphoramidate [$(R"O)_2(O)P$-], sulfenamide [$(SR")_2$-], sulfonamide [$(R"(O)_2S$-], sulfonic acid [SO_3H], phosphinamide [$(R")_2(O)P$-], sulfamoyl derivative [$R"O(O)_2S$-], thiourea [$(R")_2N(O)C$-], thiocarbamate [R"O(S)C-], phosphonate [R"-P(O)OH], amidophosphate [R"O(OH)(O)P-], carbamate (R"O(O)C-), and urea (R"NH(O)C-), wherein each R" is independently selected from the group consisting of straight or branched C_1-C_6 unsubstituted alkyl, phenyl, C_7-C_9 alkylaryl, and cycloalkyl moieties, wherein the cycloalkyl ring may span C_4-C_8 and may contain one or more heteroatoms selected from the group consisting of O,N,and S (preferred R" is selected from the group consisting of methyl, ethyl, and benzyl); and R is selected from the group consisting of straight or branched C_1 - C_6 unsubstituted alkyl, phenyl, and C_7 - C_9 alkylaryl moieties.

Preferably but optionally, the detergent compositions of this invention further comprise an effective amount of a source of calcium ions.

Preferably, the liquid detergent compositions herein comprise, by weight of composition:

a) from about 1 to about 95%, preferably from about 8% to about 70%, of said detersive surfactant;

b) from about 0.0001% to about 5%, preferably from about 0.0003% to about 0.1%, of an active proteolytic enzyme;

c) from about 0.00001% to about 5%, preferably from about 0.0001% to about 1%, more preferably from about 0.0006% to about 0.5%,of a peptide aldehyde as described hereinbefore;

d) a boron composition comprising from about 0.25% to about 10%, preferably from about 0.5% to about 5%, more preferably from about 0.75% to about 3%, by weight of boric acid or a compound capable of forming boric acid in the composition (calculated on the basis of the boric acid) and from about 1% to about 15%,

preferably from about 1.5% to about 10%, more preferably from about 2% to about 7%, by weight of the composition, of polyol, preferably in the form of a diol; and
e) from about 0.01% to about 1%, preferably from about 0.05% to about 0.5%, of calcium ion.

The proteolytic enzyme useful herein is preferably a subtilisin-type protease and may be selected from the group consisting of Alcalase®, Subtilisin BPN', Protease A, Protease B, and mixtures thereof.

The boron composition comprises boric acid or a source of boric acid ion selected from the group consisting of boric oxide, borax, other alkali metal borates, substituted boric acids; the polyol is preferably a diol and is preferably selected from 1,2 propanediol.

If used herein the source of calcium ion for use herein is preferably selected from calcium formate, calcium chloride, calcium acetate, calcium sulfate, calcium xylene sulfonate, and mixtures thereof.

Preferably, the liquid detergent compositions further comprise an effective amount one or more of the following enzymes: lipase, amylase, cellulase, and mixtures thereof. Preferably the second enzyme is lipase and is obtained by cloning the gene from Humicola Lanuginosa and expressing the gene in Aspergillus Oryzae. Lipase is utilized in an amount of from about 10 to about 18000 lipase units per gram, preferably from about from about 60 to about 6000 units per gram.

In another preferred composition herein, the second enzyme is a cellulase derived from Humicola Insolens and is utilized in an amount of from about 0.0001% to about 0.1% by weight of the total composition of said cellulase.

The compositions herein may contain further detersive adjuncts, including but not limited to, one or more of the following: suds boosters, builders, soil release polymers, polyacrylate polymers, dispersing agents, dye transfer inhibitors, dyes, perfumes, processing aids, brighteners, and mixtures thereof.

All percentages and proportions herein are by weight, and all references cited are hereby incorporated by reference, unless otherwise specifically indicated.

DETAILED DESCRIPTION OF THE INVENTION

Definitions - The present detergent compositions comprise an "effective amount" or a "stain removal-improving amount" of individual components defined herein. An "effective amount" or "stain removal-improving amount" is any amount capable of measurably improving soil cleaning or stain removal from a substrate, i.e., soiled fabric or soiled dishware, when it is washed by the consumer. In general, this amount may vary quite widely.

By "synergy" or "more than additive" as used herein is meant that the enzyme stability benefit when the calcium and peptide aldehydes are combined is greater than the sum of the individual benefits obtained when only one of the components is present in a detergent composition.

The liquid aqueous detergent compositions according to the present invention comprise four essential ingredients: (A) a peptide aldehyde or a mixture thereof, (B) a proteolytic enzyme or a mixture thereof, (C) a detersive surfactant, and (D) calcium ion. The compositions according to the present invention preferably further comprise (E) a detergent-compatible second enzyme or a mixture thereof, and may further comprise (F) other optional ingredients.

Peptide aldehydes - The detergent compositions according to the present invention comprise, as a first essential ingredient, a peptide aldehyde having the formula:

Z-B-NH-CH(R)-C(O)H

wherein B is a peptide chain comprising from 1 to 5 amino acid moieties; Z is an N-capping moiety selected from the group consisting of phosphoramidate [(R"O)$_2$(O)P-], sulfenamide [(SR")$_2$-], sulfonamide [(R"(O)$_2$S-], sulfonic acid [SO_3H], phosphinamide [(R")$_2$(O)P-], sulfamoyl derivative [R"O(O)$_2$S-], thiourea [(R")$_2$N(O)C-], thiocarbamate [R"O(S)C-], phosphonate [R"-P(O)OH], amidophosphate [R"O(OH)(O)P-], carbamate (R"O(O)C-), and urea (R"NH(O)C-), wherein each R" is independently selected from the group consisting of straight or branched C_1-C_6 unsubstituted alkyl, phenyl, C_7-C_9 alkylaryl, and cycloalkyl moieties, wherein the cycloalkyl ring may span C_4-C_8 and may contain one or more heteroatoms selected from the group consisting of O,N,and S (preferred R" is selected from the group consisting of methyl, ethyl, and benzyl); and R is selected from the group consisting of straight or branched C_1 - C_6 unsubstituted alkyl, phenyl, and C_7 - C_9 alkylaryl moieties.

Preferred R moieties are selected from the group consisting of methyl, iso-propyl, sec-butyl, iso-butyl, -C_6H_5, -CH_2-C_6H_5, and -CH_2CH_2-C_6H_5, which respectively may be derived from the amino acids Ala, Val, Ile, Leu, PGly (phenylglycine), Phe, and HPhe (homophenylalanine) by converting the carboxylic acid group to an aldehyde group. While such moieties are therefore not amino acids (and they may or may not have been synthesized from an amino acid precursor), for purposes of simplification of the exemplification of inhibitors useful here, the aldehyde portion of the inhibitors are indicated as derived from amino acids by the addition of "H" after the analogous amino acid [e.g., "-AlaH" represents the chemical moiety "-NHCH(CH_3)C(O)H"].

Preferred B peptide chains are selected from the group consisting of peptide chains having the amino acid sequences according to the general formula:

$$Z\text{-}A^5\text{-}A^4\text{-}A^3\text{-}A^2\text{-}A^1\text{-}NH\text{-}CH(R)\text{-}C(O)H$$

such that the following amino acids, when present, are :

A^1 is selected from Ala, Gly;

A^2 , if present, is selected from Val, Ala, Gly, Ile;

A^3 , if present, is selected from Phe, Leu, Val, Ile;

A^4 , if present, is any amino acid, but preferably is selected from Gly, Ala;

A^5 , if present, is any amino acid, but preferably is Gly, Ala, Lys.

The present invention aldehydes may be prepared from the corresponding amino acid whereby the C-terminal end of said amino acid is converted from a carboxylic group to an aldehyde group. Such aldehydes may be prepared by known processes, for instance as described in US 5015627, EP 185 930, EP 583,534, and DE 32 00 812.

While not wanting to be bound by theory it is believed that the peptide aldehydes according to the present invention bind to the proteolytic enzyme in the liquid detergent composition, thereby inhibiting said proteolytic enzyme. Upon dilution in water, the proteolytic activity is restored by dissociation of the proteolytic enzyme/peptide aldehyde complex.

The N-terminal end of said protease inhibitors according to the present invention is protected by one of the N-capping moiety protecting groups selected from the group consisting of carbamates, ureas, sulfonamides, phosphonamides,thioureas, sulfenamides, sulfonic acids, phosphinamides, thiocarbamates, amidophosphates, and phosphonamides. However, in a highly preferred embodiment of the present invention, the N-terminal end of said protease inhibitor is protected by a methyl, ethyl or benzyl carbamate [CH_3O-(O)C-; CH_3CH_2O-(O)C-; or $C_6H_5CH_2O$-(O)C-], methyl, ethyl or benzyl urea [CH_3NH-(O)C-; CH_3CH_2NH-(O)C-; or $C_6H_5CH_2NH$-(O)C-], methyl, ethyl or benzyl sulfonamide [CH_3SO_2-; $CH_3CH_2SO_2$-; or $C_6H_5CH_2SO_2$-], and methyl, ethyl or benzyl amidophosphate [$CH_3O(OH)(O)P$-; $CH_3CH_2O(OH)(O)P$-; or $C_6H_5CH_2O(OH)(O)P$-] groups.

Synthesis of N-capping groups can be found in the following references: <u>Protective Groups in Organic Chemistry</u>, Greene, T., Wuts, P., John Wiley & Sons, New York, 1991, pp 309-405; March, J, <u>Advanced Organic Chemistry</u>, Wiley Interscience, 1985, pp. 445, 469, Carey, F. Sundberg, R., <u>Advanced Organic Chemistry</u>, Part B, Plenum Press, New York, 1990, pp. 686-89; Atherton, E., Sheppard, R., <u>Solid Phase Peptide Synthesis</u>, Pierce Chemical, 1989, pp. 3-4; Grant,

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Examples of peptide aldehydes for use herein are: CH_3SO_2Phe-Gly-Ala-Leu-H, CH_3SO_2Val-Ala-Leu-H, $C_6H_5CH_2O(OH)(O)P$-Val-Ala-Leu-H, $CH_3CH_2SO_2$-Phe-Gly-Ala-Leu-H, $C_6H_5CH_2SO_2$-Val-Ala-Leu-H, $C_6H_5CH_2O(OH)(O)P$-Leu-Ala-Leu-H, $C_6H_5CH_2O(OH)(O)P$-Phe-Ala-Leu-H, and $CH_3O(OH)(O)P$-Leu-Gly-Ala-Leu-H.

In the Synthesis Examples hereinafter methods are disclosed to synthesize certain of these peptide aldehydes.

Synthesis Example 1

Synthesis of the tetrapeptide aldehyde Moc-Ala-Phe-Gly-Ala-LeuH

(a) Ala-Leu-OMe.HCL: To a solution of 3.0 g (14.83 mmol) Ala-Leu-OH, which is dissolved in 50 ml of MeOH and cooled to 0°C, is added 2.43 ml (33.36 mmol) thionyl chloride dropwise. This solution is stirred overnight at room temperature and evaporated to dryness providing quantitative recovery of the desired product.

(b) Cbz-Gly-Ala-Leucine methyl ester: To a solution of 0.414 g (1.98 mmol) Cbz-Gly-OH and 0.500 g (1.98 mmol) Ala-Leu-OMe.HCl in CH_2Cl_2 is add 0.607 ml TEA followed immediately by 0.355 ml DEPC. The solution is stirred overnight, evaporated, and the residue partitioned between EtOAc and 1N HCl. The organic phase is washed successively with saturated $NaHCO_3$ and saturated NaCl, dried ($MgSO_4$)and evaporated to afford 0.650 g of pure product.

(c) Moc-Ala-Phe-OH: To a solution of 1.0 g (4.23 mmol) Ala-Phe which is dissolved in 4.23 ml 1N NaOH and cooled to 0°C, 0.419 g (4.44 mmol) is aded methyl chloroformate dropwise. At the same time, in a separate addition funnel, an additional 4.23 ml 1N NaOH is added such that the pH is maintained between 9.0-9.5. After addition is complete the reaction is stirred 30 minutes at 0°C and 2 h at room temperature. At this point the solution is cooled to 0° and the pH adjusted to 9.5. This basic solution is washed with EtOAc (1X, 100 ml). The aqueous (0°C) is then adjusted to pH = 2.5 (2N HCl) and extracted with EtOAc (3X, 50 ml), dried ($MgSO_4$) and evaporated to provide 1.07 g pure product.

(d) Moc-Ala-Phe-Gly-Ala-Leu-OMe: To a solution of 0.500 g (1.22 mmol) Cbz-Gly-Ala-Leucine methyl ester in 10 ml MeOH is added 0.100 g 10% Pd/C. This solution is hydrogenated in the presence of 0.600 ml 4.0M HCl/Dioxane (under balloon pressure) for 1 h, filtered through celite and evaporated. This residue is suspended in CH_2Cl_2, 0.342 ml (2.45 mmol) TEA is added followed by 0.359 g (1.22 mmol) Moc-Ala-Phe-OH and 0.219 ml (1.34 mmol) DEPC. After stirring overnight the solvent is evaporated, the residue partitioned between EtOAc and 1N HCl and washed successively with saturated $NaHCO_3$ and NaCl. Drying, evaporation and column chromatography yield 0.450 g of the pure product.

(e) Moc-Ala-Phe-Gly-Ala-Leucinol: A solution is prepared by dissolving 0.182 g (1.64 mmol) $CaCl_2$ in a mixture of 4 ml ethanol and 2 ml THF. This mixture is cooled to -15°C and 0.450 g (0.820 mmol) Moc-Ala-Phe-Gly-Ala-Leu-OMe is added followed by 0.124 g (3.28 mmol) $NaBH_4$. The reaction is stirred for 2 h and quenched with 10 ml 1N HCl. The solvents are evaporated and the remaining aqueous layer partitioned with EtOAc. The organic phase is then washed with saturated $NaHCO_3$ and saturated NaCl. Drying ($MgSO_4$), evaporation and chromatography affords 0.256 g of pure product.

(f) Moc-Ala-Phe-Gly-Ala-LeuH: A solution is prepared by adding 0.623 g (1.47 mmol) Dess-Martin periodinane to 1.8 L CH_2Cl_2 followed by stirring for 10 minutes. This solution is then cooled to 0°C and 0.256 g (0.490 mmol) Etoc-Phe-Gly-Ala-Leucinol is added in one portion. The reaction is continued for 2 h and poured into a solution consisting of 2.55 g (10.47 mmol) $Na_2S_2O_3$ in 30 ml saturated $NaHCO_3$. After stirring for 10 minutes the mixture is extracted with EtOAc (2X, 50 ml). The combined extracts are dried ($MgSO_4$), evaporated, and chromatographed on silica to provide 0.125 g of pure product.

<u>Synthesis Example 2:</u>

<u>Synthesis of the tripeptide aldehyde Etoc-Phe-Gly-Ala-LeuH</u>

(a) Ala-Leu-OMe.HCL: To a solution of 450 g (2.20 mol) Ala-Leu-OH, which is dissolved in 4.5 L of MeOH and cooled to 0°C, is added 178.6 ml (4.95 mol) of thionyl chloride dropwise. The solution is stirred overnight at room temperature and evaporated to dryness providing 543 g (97.1% yield) of the desired product to be used as is.

(b) Etoc-Phe-Gly-OH: To a solution of 450 g (2.03 mol) Phe-Gly which is dissolved in 2026 ml 1N NaOH and cooled to 0°C, is added methyl chloroformate (3.1 ml, 40.0 mmol) dropwise. At the same time, in a separate addition funnel, an additional 2026 ml 1N NaOH is added such that the pH is maintained between 9.0-9.5. After addition is complete the reaction is stirred 30 minutes at 0°C and 2 h at room temperature. At this point the solution is cooled to 0° and the pH adjusted to 9.5. This basic solution is washed with EtOAc (1X, 4 L). The aqueous (0°C) is then adjusted to pH = 2.5 (2N HCl) and extracted with EtOAc (3X, 8L), dried ($MgSO_4$), filtered, and the solvent removed to afford 546 g (91.3% yield) pure product.

(c) Etoc-Phe-Gly-Ala-Leu-OMe: To a solution of 470 g (1.86 mol) Etoc-Phe-Gly-OH and 546 g (1.86 mol) Ala-Leu-OMe.HCl in 8 liters CH_2Cl_2 570 ml (4.09 mol) TEA is added followed by 310.4 ml (2.046 mol) DEPC. After stirring overnight the solvent is evaporated and replaced with EtOAc (4 L). This solution is washed consecutively with 2 liters each of 2N HCl, sat'd $NaHCO_3$ and sat'd NaCl. The organic phase is then dried ($MgSO_4$), filtered and evaporated to yield 916 g (93% yield) of the desired material.

(d) Etoc-Phe-Gly-Ala-Leucinol: To a solution of 45.10 g (0.406 mol) $CaCl_2$ in 1 L ethanol and 1 L THF 100 g (0.203 mol) of Etoc-Phe-Gly-Ala-Leu-OMe is added and the mixture cooled to -15°C. To this solution 30.7 g (0.812 mmol) $NaBH_4$ is carefully added followed by stirring for 2 h. Subsequently the reaction is quenched with 100ml 0.1N HCl. This solution is transfered to 4 L of 1N HCl and extracted with EtOAc (3X, 2.75 L). The combined EtOAc layers are washed with 4 L saturated $NaHCO_3$, dried ($MgSO_4$) and evaporated. Trituration (twice) with ether (4 L) provides 69.2 g (73.4% yield) of the product.

(e) Etoc-Phe-Gly-Ala-LeuH: A solution is prepared by adding 165.4 g (0.39 mol) Dess-Martin periodinane to 1.8 L CH_2Cl_2 followed by stirring for 10 minutes. This solution is then cooled to 0°C and 60 g (0.13 mol) Etoc-Phe-Gly-Ala-Leucinol added in one portion. The reaction is continued for 105 minutes and poured into a solution consisting of 6 L H_2O, 393 g $NaHCO_3$ and 431.7 g (1.74 mol) $Na_2S_2O_3$. After stirring for 10 minutes the phases are separated and 2 additional extractions (1.5 L each) with CH_2Cl_2 are performed. The combined extracts are dried ($MgSO_4$), evaporated, and triturated with (2X, 1L) ether to provide 51.7 g (86.2% yield) of the product.

Synthesis Example 3:

Synthesis of the dipeptide aldehyde Moc-Gly-Ala-LeuH

(a) Ala-Leu-OMe.HCL: To a solution of 3.0 g (14.83 mmol) Ala-Leu-OH, which is dissolved in 50 ml of MeOH and cooled to 0°C, is added 2.43 ml (33.36 mmol) of thionyl chloride dropwise. The solution is stirred overnight at room temperature and evaporated to dryness providing a quantitative yield of the desired product.

(b) Cbz-Gly-Ala-Leucine methyl ester: To a solution of 0.414 g (1.98 mmol) Cbz-Gly-OH and 0.500 g (1.98 mmol) Ala-Leu-OMe.HCl in CH_2Cl_2 0.607 ml TEA is added followed immediately by 0.355 ml DEPC. The solution is stirred overnight and then evaporated. The residue is partitioned between EtOAc and 1N HCl, the organic phase is washed with saturated $NaHCO_3$ and saturated NaCl, dried ($MgSO_4$) and evaporated providing 650 mg of pure product.

(c) Moc-Gly-Ala-Leucine methyl ester: To a solution of 2.0 g (4.90 mmol) Cbz-Gly-Ala-Leucine methyl ester which is dissolved in 20 ml MeOH is added 0.200 g 10% Pd/C. This is hydrogenated in the presence of 2.45 ml (9.81 mmol) 4.0M HCl/Dioxane for 2 h after which the reaction is thoroughly outgassed and filtered through Celite to remove the catalyst. Evaporation of the MeOH affords 1.45 g of pure product which is suspended in 45 ml CH_2Cl_2 and cooled to 0°C. To this solution 1.45 ml (3.25 mmol) TEA is added followed by 0.362 ml methyl chloroformate. After stirring overnight the CH_2Cl_2 is evaporated and the residue partitioned between EtOAc and 1N HCl. The organic phase is separated and washed sequentially with $NaHCO_3$ and NaCl. Drying, ($MgSO_4$), evaporation and chromatographic purification affords 0.820 g of desired product.

(d) Moc-Gly-Ala-Leucinol: To a solution of 0.168 g (1.51 mmol) $CaCl_2$ in 25 ml ethanol and 15 ml THF is added 0.250 g Moc-Gly-Ala-Leucine methyl ester. This solution is cooled to -15°C and 0.114 g (3.02 mmol) $NaBH_4$ is added in one portion. After stirring 2 h the reaction is quenched with 20 ml 1N HCl, concentrated on rotovape and extracted with EtOAc (2x 50ml). The combined extracts are washed with saturated $NaHCO_3$ and NaCl, dried ($MgSO_4$) and evaporated. Purification on silica provides 0.167 g of the pure product.

(e) Moc-Gly-Ala-LeuH- A solution is prepared by adding 0.418 g (0.989 mmol) Dess-Martin periodinane to 5 ml CH_2Cl_2 followed by stirring for 10 minutes. Next 0.100 g (0.330 mmol) Moc-Gly-Ala-Leucinol is added in one portion and the

reaction stirred for 2 h and poured into a 25 ml solution of saturated $NaHCO_3$ containing 1.72 g (6.93 mmol) $Na_2S_2O_3$. After stirring an additional 10 minutes the solution is extracted with EtOAc (3X, 50 ml), dried ($MgSO_4$) and evaporated. Chromatography on silica affords 0.016 g of the desired product.

<u>Synthesis Example 4:</u>

<u>Synthesis of N-(methylsulfonyl)-Phe-Gly-Ala-LeuH</u>

(a) N-Ms-Phe-Gly-OH: To a solution of 2.0 g (9.0 mmol) Phe-Gly-OH, which is dissolved in 9 ml 1N NaOH and cooled to 0°C, is added simultaneously 0.766 ml (9.9 mmol) of methane sulfonyl chloride and 9 ml 1N NaOH, in separate addition funnels. After addition is complete the reaction is stirred 15 minutes at 0°C and 1 h at room temperature. At this point the solution is cooled to 0°C, the pH adjusted to 9.5 and is washed with EtOAc (1X, 50 ml). The aqueous phase (0°C) is then adjusted to pH = 2.5 (2N HCl) and extracted with EtOAc (3X, 50 ml), dried ($MgSO_4$), filtered, and the solvent removed to afford 2.0 g pure product.

(b) N-Ms-Phe-Gly-Ala-Leucinol: A solution of is prepared by dissolving 0.500 g (1.67 mmol) N-Ms-Phe-Gly-OH in 15 ml THF, cooling to -15°C, and adding 0.366 ml (3.33 mmol) NMM followed by 0.216 ml (1.67 mmol) isobutyl chloroformate. This solution is stirred 5 minutes and 0.374 g (1.67 mmol) Ala-Leucinol.HCl, in a mixture of 10 ml THF and minimal DMF, are added. Stirring is continued at 0°C for 15 minutes and 2 h at room temperature. The solution is quenched with 5 ml 1N HCl, extracted with EtOAc (3X, 50 ml), the combined extracts are washed with sat'd $NaHCO_3$ and sat'd NaCl. The resulting organic phase is then dried ($MgSO_4$), filtered, evaporated and chromatographed on silica to yield 0.260 g of the desired material.

(c) N-Ms-Phe-Gly-Ala-LeuH: A solution is prepared by adding 0.337 g (0.798 mmol) Dess-Martin periodinane to 5 ml CH_2Cl_2 and stirring for 10 minutes. To this solution 0.125 g (0.266 mmol) N-Ms-Phe-Gly-Ala-Leucinol is added in one portion. The reaction is continued until TLC showed complete conversion at which time the solution is poured into 25 ml sat'd $NaHCO_3$ containing 1.8 g (5.586 mmol) $Na_2S_2O_3$. After stirring for 10 minutes the mixture is extracted with EtOAc (3X, 50 ml). The combined extracts are dried ($MgSO_4$), evaporated, and chromatographed on silica to afford 0.048 g of the product.

<u>Synthesis Example 5:</u>

Synthesis of an aldehyde protease inhibitor

Moc-Leu-OH-L-Leucine (5.0 g, 38.2 mmol) is dissolved in 38 ml 1N NaOH and cooled to 0°C. Methyl chloroformate (3.1 ml, 40.0 mmol) is added dropwise while in a separate addition funnel 1N NaOH is added as to maintain pH at 9.0-9.5. After addition is complete and the pH stabilized at 9.0-9.5 the solution is washed with 200 ml EtOAc, the aqueous phase is then acidified to pH = 2. This mixture is extracted with EtOAc (2X 100 ml), dried ($MgSO_4$), filtered, and the solvent removed to afford 7.15 g pure product.

Moc-Leu-Leucinol- To a solution of 3.5 g (18.52 mmol) Moc-Leu-OH in 100 ml THF, cooled to -15°C, 2.04 ml (18.52 mmol) of N-methyl morpholine is added followed immediatedly by 2.4 ml (18.52 mmol) isobutyl chloroformate. After stirring for 10 minutes 2.37 ml (18.52 mmol) of leucinol in 25 ml of THF is added and the reaction stirred 0.5 h at -15°C and 1 h at room temperature. The mixture is then diluted with 100 ml of H_20 and the THF evaporated. The remaining aqueous phase is partitioned between EtOAc and 1N HCl, the organic phase washed with $NaHCO_3$, dried ($MgSO_4$) and evaporated to afford 5.33 g pure product.

Moc-Leu-LeuH-A solution containing 4.4 g (10.41 mmol) Dess-Martin periodinane suspended in 100 ml CH_2Cl_2 is prepared and stirred for 10 minutes. To this solution 1.0 g (3.47 mmol) Moc-Leu-Leucinol is added and the solution stirred 2 h at room temperature followed by pouring into 100 ml of saturated $NaHCO_3$ containing 18 g (72.87 mmol) $Na_2S_2O_3$. This solution is stirred 10 minutes and then extracted with EtOAc (2X, 125ml), dried ($MgSO_4$) and the solvent evaporated. Chromatography on silica affords 0.550 g of pure product.

Synthesis Example 6:

Additional peptide aldehydes are synthesized according to the following procedures. Some of the intermediates are purchased from suppliers and in these instances it is noted within the procedure. Dess-Martin periodinane is synthesized according to the procedure of Martin, J.Org. Chem., 1983, 48, 4155.

I. Z-Gly-Ala-Leu-OMe - To a solution of Z-Gly-Ala-OH (20.0 g, 0.071 M) and Leu-OMe.HCl (12.9 g, 0.071 M) in 250 ml dichloromethane is added 21.9 ml (0.157 M) triethylamine (TEA) dropwise over a period of 10 min. This addition is followed by the addition of 11.9 ml (0.078 M) of diethylcyanophosphonate (DECP). The mixture is stirred overnight and the solvent removed. The residue is dissolved in

ethyl acetate and washed with 1N HCl, saturated $NaHCO_3$, and brine. The solution is dried with $MgSO_4$, filtered and the solvent removed. Recovered will be 29.0 g of product that is homogeneous by TLC. ^{13}C NMR ($CDCl_3$) 15.93, 18.60, 21.77, 22.69, 24.72, 40.80, 44.20, 48.70, 50.87, 52.13, 65.28, 66.84, 127.92, 128.00, 128.41, 136.36, 156.76, 169.31, 172.58, 173.24.

II. Moc-Phe-Gly-Ala-Leu-OMe - Z-Gly-Ala-Leu-OMe (29.0 g, 0.071 M) is dissolved in 300 ml MeOH and 35 ml 4.0 M HCl in dioxane. To this solvent mixture is added 5.8 g of 10% Pd/C portionwise. The slurry is degassed with an aspirator and H_2 introduced via balloon. The slurry is maintained under a positive pressure of H_2 and stirred overnight. The slurry is filtered through Celite and a sintered glass funnel and washed thoroughly with MeOH. The solvent is removed and the residue is triturated with ether. The slurry is filtered and the filter cake dried under vacuum. Recovered 20.2 g of an off-white powder. The crude product and Moc-Phe-OH (15.3 g, 0.068 M) are dissolved in 500 ml CH_2Cl_2 and 29.9 ml TEA (0.143 M) added dropwise followed by the dropwise addition of 11.7 ml (0.072 M) of DECP. The mixture iss stirred overnight and the solvent is removed. The residue is dissolved in EtOAc and washed with 1N HCl, saturated $NaHCO_3$, and brine. The organic phase is dried ($MgSO_4$), filtered and the solvent removed to afford 21.3 g product. ^{13}C NMR ($CDCl_3$) 16.66, 16.83, 20,01, 22.46, 23.41, 25.40, 40.11, 41.72, 43.75, 49.39, 51.37, 52.87, 56.42, 65.92, 77.39, 77.55, 77.81, 78.24, 127.42, 128.96, 129.19, 130.09, 137.41, 157.62, 169.00, 172.63, 173.24, 174.00.

III. Moc-Phe-Gly-Ala-Leucinol - Moc-Phe-Gly-Ala-Leu-OMe (21.3 g, 44.5 mmol) is dissolved in a mixture of 400 ml EtOH and 250 ml THF. The solution is cooled to 0°C and 9.88 g (89.0 mmol) $CaCl_2$ is added. In 5 min the slurry will be homogenized and 6.73 g (178.0 mmol) $NaBH_4$ added portionwise over a period of 5 min. The solution is stirred at 0°C for 2 hours and the reaction carefully quenched with 1N HCl. The EtOH and THF are removed under vacuum and the remaining aqueous mixture extracted with 500 ml EtOAc. This organic phase is washed with saturated $NaHCO_3$, brine, and the organic phase dried with $MgSO_4$. Filtration and removal of solvent affords 20.0 g of an off-white crystalline material. Chromatography on silica (3.5% $MeOH/CH_2Cl_2$) gives 13.0 g pure product. R_f = 0.3 (10% $MeOH/CH_2Cl_2$), ^{13}C NMR ($CDCl_3$) 17.50, 22.23, 23.12, 24.84, 37.22, 39.76, 43.96, 49.88, 50.93, 52.48, 58.22, 65.27, 98.46, 98.54, 127.04, 128.68, 129.10, 136.62, 157.85, 170.71, 173.85, 174.45

IV. Moc-Phe-Gly-Ala-Leu-H - 29.9 g (70.7 mmol) of Dess-Martin periodinane is suspended in 500 ml CH_2Cl_2 and stirred for 10 min. Moc-Phe-Gly-Ala-Leucinol (10.6 g, 23.5 mmol) iss dissolved in 100 ml CH_2Cl_2 and added at a moderate rate to the periodinane slurry. The mixture is stirred for 1h and poured into 150 ml $NaHCO_3$ containing 123 g $Na_2S_2O_3$. The mixture is allowed to stir for 15 min and extracted with EtOAc. The organic phase is dried and filtered followed by removal of solvent. Chromatography (3.5% MeOH/CH_2Cl_2) on silica gives 5.1 g of pure white solid that is a mixture of the methoxy hemiacetal and aldehyde. ^{13}C NMR ($CDCl_3$,CD_3OD) 17.62, 17.94, 21.53, 21.71, 22.99, 23.30, 23.39, 24.54, 37.05, 37.70, 37.92, 38.24, 42.87, 49.83, 51.79, 52.14, 52.40, 56.75, 57.19, 98.40, 99.18, 127.00, 128.60, 129.06, 136.44, 157.27, 169.19, 169.67, 172.73, 173.40, 200.43.

V. Moc-Phe-OH - L-Phenylalanine (5.0 g, 30.2 mmol) is dissolved in 30 ml 1N NaOH and cooled to 0°C. Methyl chloroformate (2.53 ml, 31.8 mmol) is added dropwise while in a separate addition funnel 30 ml of 1N NaOH is added simultaneously. After addition is complete, the solution is washed with 200 ml EtOAc and the aqueous phase acidified to pH = 2. The mixture is extracted with EtOAc (2X 100 ml), dried ($MgSO_4$), filtered, and the solvent removed to afford 6.0 g product. ^{13}C NMR ($CDCl_3$) 37.75, 52.57, 54.64, 128.63, 129.35, 135.74, 156.77, 175.76.

VI. Mac-Phe-OH - To a solution of 1.00 g (2.34 mmol) of Phe-OBn.PTSA in Et_2O at room temperature is added 0.36 ml (2.57 mmol) of TEA. This is followed by the addition of 10 ml MeOH and then 0.14 ml (2.34 mmol) of methyl isocyanate in 4 ml Et_2O is added dropwise. The reaction mixture is poured into 50 ml water and the phases separate. The organic phase is dried with $MgSO_4$, filtered and the solvent removed to give 0.66 g of product (96% yield). ^{13}C NMR ($CDCl_3$) 27.05, 38.47, 53.45, 54.64, 65.90, 127.43, 127.85, 128.48, 129.28, 130.27, 135.23, 136.22, 158.17, 173.08. To a solution of the crude product (2.11 mmol) in 25 ml MeOH is added 0.120 g Pd/C and the slurry degassed. The slurry is stirred under a positive pressure of H_2 via balloon for 1.5 h. The slurry is filtered through Celite and the filter cake washed with MeOH. The solvent is removed to afford O.430 g product. ^{13}C NMR 26.50, 37.92, 54.28, 126.69, 128.28, 129.28, 136.65, 159.36, 175.33.

VII. Mac-Phe-Gly-Ala-Leucinol - To a solution of 0.200 g Mac-Phe-OH (0.900 mmol) and 0.253 g Gly-Ala-Leu-OMe.HCl (0.818 mmol, generated by hydrogenation of I., above, according to the procedure outlined for compound II.) in

15 ml DMF is added 0.250 ml TEA (1.80 mmol) followed by the addition of 0.147 ml DECP (0.900 mmol). The mixture is stirred overnight and the solvent removed. The residue is redissolved in EtOAc and washed successively with 0.3 N HCl, saturated $NaHCO_3$, and brine. The solution is dried, filtered and the solvent removed to give 0.300 g product. The crude product (0.628 mmol) is dissolved in 17 ml EtOH and cooled to 0°C. To this solution is added 0.140 g $CaCl_2$ (1.25 mmol) in 4 ml THF. To the resulting slurry is added 0.095 g $NaBH_4$ in one portion. After 45 min. the solution is quenched with water and extracted with EtOAc. The organic phase is dried with $MgSO_4$, filtered and the solvent removed. Chromatography with 4% $MeOH/CH_2Cl_2$ gave 0.200 g pure product. ^{13}C NMR (CD_3OD) 16.84, 21.05, 22.60, 24.51, 25.66, 37.41, 39.73, 42.67, 49.65, 56.63, 64.33, 126.63, 128.32, 128.96, 137.12, 160.01, 170.45, 173.60, 175.03.

VIII. Mac-Phe-Gly-Ala-Leu-H - To a slurry of Dess-Martin periodinane (0.565 g, 1.33 mmol) in 15 ml CH_2Cl_2 is added a suspension of Mac-Phe-Gly-Ala-Leucinol (0.200 g, 0.445 mmol) in CH_2Cl_2 and the resulting slurry stirred for 0.5 h. The mixture is poured into saturated $NaHCO_3$ containing 2.32 g $Na_2S_2O_3$ and the solution stirred for 10 min., followed by extraction with EtOAc. The organic phase is dried with $MgSO_4$, filtered and the solvent removed. The residue is chromatographed on silica to give 0.081 g product. ^{13}C NMR (10% CD_3OD in $CDCl_3$) 17.18, 17.43, 21.35, 21.55, 23.26, 23.34, 24.40, 24.47, 26.36, 26.60, 37.25, 37.38, 38.60, 42.86, 42.97, 51.77, 51.93, 54.94, 56.75, 57.00, 98.7, 99.32, 126.87, 128.49, 128.91, 136.51, 159.53, 159.55, 169.93, 170.39, 173.63, 173.85, 174.70.

Cbz = carbobenzyloxy
Gly = glycine
Ala = alanine
Leu = leucine
Phe = phenylalanine
OMe = methyl ester
TEA = triethylamine
DECP = diethylcyanophosphonate
TLC = thin layer chromatography
MeOH = methanol
Pd/C = palladium on activated carbon
EtOH = ethanol
THF = tetrahydrofuran

Mac = methylaminocarbonyl
Moc = methoxycarbonyl
Etoc = ethoxycarbonyl
Ms = methanesulfonyl

Proteolytic Enzyme - Another essential ingredient in the present liquid detergent compositions is active proteolytic enzyme. Mixtures of proteolytic enzyme are also included. The proteolytic enzyme can be of animal, vegetable or microorganism (preferred) origin. The proteases for use in the detergent compositions herein include (but are not limited to) trypsin, subtilisin, chymotrypsin and elastase-type proteases. Preferred for use herein are subtilisin-type proteolytic enzymes. Particularly preferred is bacterial serine proteolytic enzyme obtained from Bacillus subtilis and/or Bacillus licheniformis. Protease enzymes are usually present in such liquid detergent compositions at levels sufficient to provide from 0.005 to 0.1 Anson units (AU) of activity per gram of composition.

Suitable proteolytic enzymes include Novo Industri A/S Alcalase® (preferred), Esperase® , Savinase® (Copenhagen, Denmark), Gist-brocades' Maxatase®, Maxacal® and Maxapem 15® (protein engineered Maxacal®) (Delft, Netherlands), and subtilisin BPN and BPN'(preferred), which are commercially available. Preferred proteolytic enzymes are also modified bacterial serine proteases, such as those made by Genencor International, Inc.(San Francisco, California) which are described in European Patent 251,446, filed April 28, 1987 (particularly pages 17, 24 and 98), and which is called herein "Protease B", and U.S. Patent 5,030,378, Venegas, issued July 9, 1991, which refers to a modified bacterial serine proteolytic enzyme (Genencor International) which is called "Protease A" herein (same as BPN'). In particular see columns 2 and 3 of U.S. Patent 5,030,378 for a complete description, including amino sequence, of Protease A and its variants. Preferred proteolytic enzymes, then, are selected from the group consisting of Alcalase ® (Novo Industri A/S), BPN', Protease A and Protease B (Genencor), and mixtures thereof. Protease B is most preferred.

Another preferred protease, referred to as "Protease D" is a carbonyl hydrolase variant having an amino acid sequence not found in nature, which is derived from a precursor carbonyl hydrolase by substituting a different amino acid for a plurality of amino acid residues at a position in said carbonyl hydrolase equivalent to position +76, preferably also in combination with one or more amino acid residue positions equivalent to those selected from the group consisting of +99, +101, +103, +104, +107, +123, +27, +105, +109, +126, +128, +135, +156, +166,

+195, +197, +204, +206, +210, +216, +217, +218, +222, +260, +265, and/or +274 according to the numbering of *Bacillus amyloliquefaciens* subtilisin, as described in WO 95/10615 published April 20, 1995 by Genencor International.

Useful proteases are also described in PCT publications: WO 95/30010 published Novenber 9, 1995 by The Procter & Gamble Company; WO 95/30011 published Novenber 9, 1995 by The Procter & Gamble Company; WO 95/29979 published Novenber 9, 1995 by The Procter & Gamble Company.

Calcium - If used herein, any water-soluble calcium salt can be used as a source of calcium ions, including calcium acetate, calcium formate, calcium xylene sulfonate, and calcium propionate. Divalent ions, such as zinc and magnesium ions, can replace the calcium ion completely or in part. Thus for the liquid detergent compositions herein, the source of calcium ions can be partially or completely substituted with a source of another divalent ion.

The calcium useful herein is enzyme-accessible. Therefore, the claimed compositions are substantially free of sequestrants, for example, polyacids capable of forming calcium complexes which are soluble in the composition. However, minor amounts of sequestrants such as polyacids or mixtures of polyacids can be used. The enzyme-accessible calcium is defined as the amount of calcium-ions effectively available to the enzyme component. From a practical standpoint the enzyme-accessible calcium is therefore the soluble calcium in the composition in the absence of any storage sequestrants, e.g., having an equilibrium constant of complexation with calcium equal to or greater than 1.5 at 20^{o}C.

Boric Acid - The compositions herein contain from about 0.25% to about 10%, preferably from about 0.5% to about 5%, more preferably from about 0.75% to about 3%, by weight of boric acid or a compound capable of forming boric acid in the composition (calculated on the basis of the boric acid). Boric acid is preferred, although other compounds such as boric oxide, borax and other alkali metal borates (e.g., sodium ortho-, meta-, pyroborate, and sodium pentaborate) are suitable. Substituted boric acids (e.g., phenylboronic acid, butane boronic acid, and p-bromo phenylboronic acid) can also be used in place of boric acid.

The compositions of the present invention also contain polyols, especially diols, containing only carbon, hydrogen and oxygen atoms. They preferably contain from about 2 to about 6 hydroxy groups. Examples include propylene glycol (especially 1,2 propanediol, which is preferred), ethylene glycol, glycerol, sorbitol, mannitol, glucose, and mixtures thereof. The polyol generally represents from about 1% to about 15%, preferably from about 1.5% to about 10%, more preferably from about 2% to about 7%, by weight of the composition.

Detersive Surfactant - An effective amount, typically from about 1 to 95, preferably about 8 to 70, weight %, of detersive surfactant is yet another essential ingredient in the present invention. The detersive surfactant can be selected from the group consisting of anionics, nonionics, cationics, ampholytics, zwitterionics, and mixtures thereof. By selecting the type and amount of detersive surfactant, along with other adjunct ingredients disclosed herein, the present detergent compositions can be formulated to be used in the context of laundry cleaning or in other different cleaning applications, particularly including dishwashing. The particular surfactants used can therefore vary widely depending upon the particular end-use envisioned.

The benefits of the present invention are especially pronounced in compositions containing ingredients that are harsh to enzymes such as certain detergency builders and surfactants. These include (but are not limited to) anionic surfactants such as alkyl ether sulfate linear alkyl benzene sulfonate, alkyl sulfate, etc. Suitable surfactants are described below.

Anionic Surfactants - One type of anionic surfactant which can be utilized encompasses alkyl ester sulfonates. These are desirable because they can be made with renewable, non-petroleum resources. Preparation of the alkyl ester sulfonate surfactant component can be effected according to known methods disclosed in the technical literature. For instance, linear esters of C_8-C_{20} carboxylic acids can be sulfonated with gaseous SO_3 according to "The Journal of the American Oil Chemists Society," 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm, and coconut oils, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprises alkyl ester sulfonate surfactants of the structural formula:

$$R^3\text{-}\underset{\displaystyle SO_3M}{CH}\text{-}\overset{\displaystyle O}{C}\text{-}OR^4$$

wherein R^3 is a C_8-C_{20} hydrocarbyl, preferably an alkyl, or combination thereof, R^4 is a C_1-C_6 hydrocarbyl, preferably an alkyl, or combination thereof, and M is a soluble salt-forming cation. Suitable salts include metal salts such as sodium, potassium, and lithium salts, and substituted or unsubstituted ammonium salts, such as methyl-, dimethyl, -trimethyl, and quaternary ammonium cations, e.g. tetramethyl-ammonium and dimethyl piperdinium, and cations derived from alkanolamines, e.g. monoethanol-amine, diethanolamine, and triethanolamine. Preferably, R^3 is C_{10}-C_{16} alkyl, and R^4 is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein R^3 is C_{14}-C_{16} alkyl.

Alkyl sulfate surfactants are another type of anionic surfactant of importance for use herein. In addition to providing excellent overall cleaning ability when used in combination with polyhydroxy fatty acid amides (see below), including good grease/oil cleaning over a wide range of temperatures, wash concentrations, and wash times, dissolution of alkyl sulfates can be obtained, as well as improved formulability in liquid detergent formulations are water soluble salts or acids of the formula $ROSO_3M$ wherein R preferably is a C_{10}-C_{24} hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C_{10}-C_{20} alkyl component, more preferably a C_{12}-C_{18} alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g., sodium, potassium, lithium), substituted or unsubstituted ammonium cations such as methyl-, dimethyl-, and trimethyl ammonium and quaternary ammonium cations, e.g., tetramethyl-ammonium and dimethyl piperdinium, and cations derived from alkanolamines such as ethanolamine, diethanolamine, triethanolamine, and mixtures thereof, and the like. Typically, alkyl chains of C_{12-16} are preferred for lower wash temperatures (e.g., below about 50°C) and C_{16-18} alkyl chains are preferred for higher wash temperatures (e.g., above about 50°C).

Alkyl alkoxylated sulfate surfactants are another category of useful anionic surfactant. These surfactants are water soluble salts or acids typically of the formula $RO(A)_mSO_3M$ wherein R is an unsubstituted C_{10}-C_{24} alkyl or hydroxyalkyl group having a C_{10}-C_{24} alkyl component, preferably a C_{12}-C_{20} alkyl or hydroxyalkyl, more preferably C_{12}-C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl-, trimethyl-ammonium and quaternary ammonium cations, such as tetramethyl-ammonium, dimethyl piperidinium and cations derived from alkanolamines, e.g. monoethanolamine, diethanolamine, and triethanolamine, and mixtures thereof. Exemplary surfactants are C_{12}-C_{18} alkyl polyethoxylate (1.0) sulfate, C_{12}-C_{18} alkyl polyethoxylate (2.25) sulfate, C_{12}-C_{18} alkyl polyethoxylate (3.0) sulfate, and C_{12}-C_{18} alkyl polyethoxylate (4.0) sulfate wherein M is conveniently selected from sodium and potassium.

Other Anionic Surfactants - Other anionic surfactants useful for detersive purposes can also be included in the compositions hereof. These can include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium

salts such as mono-, di- and triethanolamine salts) of soap, C_9-C_{20} linear alkylbenzenesulphonates, C_8-C_{22} primary or secondary alkanesulphonates, C_8-C_{24} olefinsulphonates, sulphonated polycarboxylic acids prepared by sulphonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isothionates such as the acyl isothionates, N-acyl taurates, fatty acid amides of methyl tauride, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinate (especially saturated and unsaturated C_{12}-C_{18} monoesters) diestèrs of sulfosuccinate (especially saturated and unsaturated C_6-C_{14} diesters), N-acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, alkyl polyethoxy carboxylates such as those of the formula $RO(CH_2CH_2O)_kCH_2COO\text{-}M^+$ wherein R is a C_8-C_{22} alkyl, k is an integer from 0 to 10, and M is a soluble salt-forming cation, and fatty acids esterified with isethionic acid and neutralized with sodium hydroxide. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil. Further examples are given in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perry and Berch). A variety of such surfactants are also generally disclosed in U.S. Patent 3,929,678, issued December 30, 1975 to Laughlin, et al. at Column 23, line 58 through Column 29, line 23 (herein incorporated by reference).

Nonionic Detergent Surfactants - Suitable nonionic detergent surfactants are generally disclosed in U.S. Patent 3,929,678, Laughlin et al., issued December 30, 1975, at column 13, line 14 through column 16, line 6, incorporated herein by reference. Exemplary, non-limiting classes of useful nonionic surfactants are listed below.

The polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols. In general, the polyethylene oxide condensates are preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to about 12 carbon atoms in either a straight chain or branched chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount equal to from about 5 to about 25 moles of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include Igepal® CO-630, marketed by the GAF Corporation; and Triton® X-45, X-114, X-100, and X-102, all marketed by the Rohm & Haas

Company. These compounds are commonly referred to as alkyl phenol alkoxylates, (e.g., alkyl phenol ethoxylates).

The condensation products of aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Particularly preferred are the condensation products of alcohols having an alkyl group containing from about 10 to about 20 carbon atoms with from about 2 to about 18 moles of ethylene oxide per mole of alcohol. Examples of commercially available nonionic surfactants of this type include Tergitol® 15-S-9 (the condensation product of C_{11}-C_{15} linear secondary alcohol with 9 moles ethylene oxide), Tergitol® 24-L-6 NMW (the condensation product of C_{12}-C_{14} primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; Neodol® 45-9 (the condensation product of C_{14}-C_{15} linear alcohol with 9 moles of ethylene oxide), Neodol® 23-6.5 (the condensation product of C_{12}-C_{13} linear alcohol with 6.5 moles of ethylene oxide), Neodol® 45-7 (the condensation product of C_{14}-C_{15} linear alcohol with 7 moles of ethylene oxide), Neodol® 45-4 (the condensation product of C_{14}-C_{15} linear alcohol with 4 moles of ethylene oxide), marketed by Shell Chemical Company, and Kyro® EOB (the condensation product of C_{13}-C_{15} alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company. This category of nonionic surfactant is referred to generally as "alkyl ethoxylates."

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol. The hydrophobic portion of these compounds preferably has a molecular weight of from about 1500 to about 1800 and exhibits water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide. Examples of compounds of this type include certain of the commercially-available Pluronic® surfactants, marketed by BASF.

The condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. The hydrophobic moiety of these products consists of the reaction product of ethylenediamine and excess propylene oxide, and generally has a molecular weight of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of

polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic® compounds, marketed by BASF.

Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; water-soluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula

$$R^3(OR^4)_x\overset{O}{N}(R^5)_2$$

wherein R^3 is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; R^4 is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3; and each R^5 is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The R^5 groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure.

These amine oxide surfactants in particular include C_{10}-C_{18} alkyl dimethyl amine oxides and C_8-C_{12} alkoxy ethyl dihydroxy ethyl amine oxides.

Alkylpolysaccharides disclosed in U.S. Patent 4,565,647, Llenado, issued January 21, 1986, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharide, e.g., a polyglycoside, hydrophilic group containing from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moieties. (Optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside.) The intersaccharide bonds can be, e.g., between the one position of the

additional saccharide units and the 2-, 3-, 4-, and/or 6- positions on the preceding saccharide units.

Optionally, and less desirably, there can be a polyalkylene-oxide chain joining the hydrophobic moiety and the polysaccharide moiety. The preferred alkyleneoxide is ethylene oxide. Typical hydrophobic groups include alkyl groups, either saturated or unsaturated, branched or unbranched containing from about 8 to about 18, preferably from about 10 to about 16, carbon atoms. Preferably, the alkyl group is a straight chain saturated alkyl group. The alkyl group can contain up to about 3 hydroxy groups and/or the polyalkyleneoxide chain can contain up to about 10, preferably less than 5, alkyleneoxide moieties. Suitable alkyl polysaccharides are octyl, nonyl, decyl, undecyldodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, and octadecyl, di-, tri-, tetra-, penta-, and hexaglucosides, galactosides, lactosides, glucoses, fructosides, fructoses and/or galactoses. Suitable mixtures include coconut alkyl, di-, tri-, tetra-, and pentaglucosides and tallow alkyl tetra-, penta-, and hexa-glucosides.

The preferred alkylpolyglycosides have the formula

$$R^2O(C_nH_{2n}O)_t(\text{glycosyl})_x$$

wherein R^2 is selected from the group consisting of alkyl, alkyl-phenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-position and the preceding glycosyl units 2-, 3-, 4- and/or 6-position, preferably predominantly the 2-position.

Fatty acid amide surfactants having the formula:

$$R^6-\overset{\displaystyle O}{\overset{\|}{C}}-N(R^7)_2$$

wherein R^6 is an alkyl group containing from about 7 to about 21 (preferably from about 9 to about 17) carbon atoms and each R^7 is selected from the group consisting of hydrogen, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, and $-(C^2H_4O)_xH$ where x varies from about 1 to about 3.

Preferred amides are C_8-C_{20} ammonia amides, monoethanolamides, diethanolamides, and isopropanolamides.

Cationic Surfactants - Cationic detersive surfactants can also be included in detergent compositions of the present invention. Cationic surfactants include the ammonium surfactants such as alkyldimethylammonium halogenides, and those surfactants having the formula:

$$[R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N^+X^-$$

wherein R^2 is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each R^3 is selected from the group consisting of $-CH_2CH_2-$, $-CH_2CH(CH_3)-$, $-CH_2CH(CH_2OH)-$, $-CH_2CH_2CH_2-$, and mixtures thereof; each R^4 is selected from the group consisting of C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, benzyl, ring structures formed by joining the two R^4 groups, -$CH_2CHOHCHOHCOR^6CHOH$-CH_2OH wherein R^6 is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not O; R^5 is the same as R^4 or is an alkyl chain wherein the total number of carbon atoms of R^2 plus R^5 is not more than about 18; each y is from 0 to about 10 and the sum of the y values is from 0 to about 15; and X is any compatible anion.

Other cationic surfactants useful herein are also described in U.S. Patent 4,228,044, Cambre, issued October 14, 1980, incorporated herein by reference.

Other Surfactants - Ampholytic surfactants can be incorporated into the detergent compositions hereof. These surfactants can be broadly described as aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight chain or branched. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic water-solubilizing group, e.g., carboxy, sulfonate, sulfate. See U.S. Patent No. 3,929,678 to Laughlin et al., issued December 30, 1975 at column 19, lines 18-35 for examples of ampholytic surfactants.

Zwitterionic surfactants can also be incorporated into the detergent compositions hereof. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. See U.S. Patent No. 3,929,678 to Laughlin et al., issued December 30, 1975 at column 19, line 38 through column 22, line 48 for examples of zwitterionic surfactants. Ampholytic and zwitterionic surfactants are generally used in combination with one or more anionic and/or nonionic surfactants.

Polyhydroxy Fatty Acid Amide Surfactant - The liquid detergent compositions hereof may also contain an enzyme-enhancing amount of polyhydroxy fatty acid amide surfactant. By "enzyme-enhancing" is meant that the formulator of the

composition can select an amount of polyhydroxy fatty acid amide to be incorporated into the compositions that will improve enzyme cleaning performance of the detergent composition. In general, for conventional levels of enzyme, the incorporation of about 1%, by weight, polyhydroxy fatty acid amide will enhance enzyme performance.

The detergent compositions herein will typically comprise about 1% weight basis, polyhydroxy fatty acid amide surfactant, preferably from about 3% to about 30%, of the polyhydroxy fatty acid amide. The polyhydroxy fatty acid amide surfactant component comprises compounds of the structural formula:

$$\begin{array}{ccccc} & \mathrm{O} & & R^1 & \\ R^2 - & \mathrm{C} & - & \mathrm{N} & - \mathrm{Z} \end{array}$$

wherein: R^1 is H, C_1-C_4 hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl, or a mixture thereof, preferably C_1-C_4 alkyl, more preferably C_1 or C_2 alkyl, most preferably C_1 alkyl (i.e., methyl); and R^2 is a C_5-C_{31} hydrocarbyl, preferably straight chain C_7-C_{19} alkyl or alkenyl, more preferably straight chain C_9-C_{17} alkyl or alkenyl, most preferably straight chain C_{11}-C_{15} alkyl or alkenyl, or mixtures thereof; and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative (preferably ethoxylated or propoxylated) thereof. Z preferably will be derived from a reducing sugar in a reductive amination reaction; more preferably Z will be a glycityl. Suitable reducing sugars include glucose, fructose, maltose, lactose, galactose, mannose, and xylose. As raw materials, high dextrose corn syrup, high fructose corn syrup, and high maltose corn syrup can be utilized as well as the individual sugars listed above. These corn syrups may yield a mix of sugar components for Z. It should be understood that it is by no means intended to exclude other suitable raw materials. Z preferably will be selected from the group consisting of $-CH_2-(CHOH)_n-CH_2OH$, $-CH(CH_2OH)-(CHOH)_{n-1}-CH_2OH$, $-CH_2-(CHOH)_2(CHOR')(CHOH)-CH_2OH$, and alkoxylated derivatives thereof, where n is an integer from 3 to 5, inclusive, and R' is H or a cyclic or aliphatic monosaccharide. Most preferred are glycityls wherein n is 4, particularly $-CH_2-(CHOH)_4-CH_2OH$.

R' can be, for example, N-methyl, N-ethyl, N-propyl, N-isopropyl, N-butyl, N-2-hydroxy ethyl, or N-2-hydroxy propyl.

R^2-CO-N< can be, for example, cocamide, stearamide, oleamide, lauramide, myristamide, capricamide, palmitamide, tallowamide, etc.

Z can be 1-deoxyglucityl, 2-deoxyfructityl, 1-deoxymaltityl, 1-deoxylactityl, 1-deoxygalactityl, 1-deoxymannityl, 1-deoxymaltotriotityl, etc.

Methods for making polyhydroxy fatty acid amides are known in the art. In general, they can be made by reacting an alkyl amine with a reducing sugar in a reductive amination reaction to form a corresponding N-alkyl polyhydroxyamine, and then reacting the N-alkyl polyhydroxyamine with a fatty aliphatic ester or triglyceride in a condensation/amidation step to form the N-alkyl, N-polyhydroxy fatty acid amide product. Processes for making compositions containing polyhydroxy fatty acid amides are disclosed, for example, in G.B. Patent Specification 809,060, published February 18, 1959, by Thomas Hedley & Co., Ltd., U.S. Patent 2,965,576, issued December 20, 1960 to E. R. Wilson, and U.S. Patent 2,703,798, Anthony M. Schwartz, issued March 8, 1955, and U.S. Patent 1,985,424, issued December 25, 1934 to Piggott, each of which is incorporated herein by reference.

<u>Second Enzyme</u> - Preferred compositions herein further comprise a performance-enhancing amount of a detergent-compatible second enzyme. By "detergent-compatible" is meant compatibility with the other ingredients of a liquid detergent composition, such as detersive surfactant and detergency builder. These second enzymes are preferably selected from the group consisting of lipase, amylase, cellulase, and mixtures thereof. The term "second enzyme" excludes the proteolytic enzymes discussed above, so each composition which has a second enzyme contains at least two kinds of enzyme, including at least one proteolytic enzyme. The amount of second enzyme used in the composition varies according to the type of enzyme. In general, from about 0.0001 to 0.3, more preferably 0.001 to 0.1, weight % of these second enzymes are preferably used. Mixtures of the same class of enzymes (e.g. lipase) or two or more classes (e.g. cellulase and lipase) may be used. Purified or non-purified forms of the enzyme may be used.

Any lipolytic enzyme suitable for use in a liquid detergent composition can be used in these compositions. Suitable lipase enzymes for use herein include those of bacterial and fungal origin.

Suitable bacterial lipases include those produced by microorganisms of the <u>Pseudomonas</u> groups, such as <u>Pseudomonas</u> <u>stutzeri</u> ATCC 19.154, as disclosed in British Patent 1,372,034, incorporated herein by reference. Suitable lipases include those which show a positive immunological cross-reaction with the antibody of the lipase produced by the microorganism <u>Pseudomonas</u> <u>fluorescens</u> IAM 1057. This lipase and a method for its purification have been described in Japanese Patent Application 53-20487, laid open on February 24, 1978. This lipase is available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan, under the trade name Lipase P "Amano," hereinafter referred to as "Amano-P." Such lipases should show a

positive immunological cross-reaction with the Amano-P antibody, using the standard and well-known immunodiffusion procedure according to Ouchterlony (Acta. Med. Scan., 133, pages 76-79 (1950)). These lipases, and a method for their immunological cross-reaction with Amano-P, are also described in U.S. Patent 4,707,291, Thom et al., issued November 17, 1987, incorporated herein by reference. Typical examples thereof are the Amano-P lipase, the lipase ex Pseudomonas fragi FERM P 1339 (available under the trade name Amano-B), lipase ex Pseudomonas nitroreducens var. lipolyticum FERM P 1338 (available under the trade name Amano-CES), lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRLB 3673, commercially available from Toyo Jozo Co., Tagata, Japan; and further Chromobacter viscosum lipases from U.S. Biochemical Corp., U.S.A. and Disoynth Co., The Netherlands, and lipases ex Pseudomonas gladioli.

Suitable fungal lipases include those producible by Humicola lanuginosa and Thermomyces lanuginosus. Most preferred is lipase obtained by cloning the gene from Humicola lanuginosa and expressing the gene in Aspergillus oryzae as described in European Patent Application 0 258 068 (Novo Industri A/S), commercially available from Novo Nordisk A/S under the trade name Lipolase®.

From about 10 to 18,000, preferably about 60 to 6,000, lipase units per gram (LU/g) of lipase can be used in these compositions. A lipase unit is that amount of lipase which produces 1 mmol of titratable fatty acid per minute in a pH stat, where pH is 9.0, temperature is 30°C, substrate is an emulsion of 3.3wt % of olive oil and 3.3% gum arabic, in the presence of 13 mmol/l Ca^{++} and 20 mmol/l NaCl in 5 mmol/l Tris-buffer.

Any cellulase suitable for use in a liquid detergent composition can be used in these compositions. Suitable cellulase enzymes for use herein include those from bacterial and fungal origins. Preferably, they will have a pH optimum of between 5 and 9.5. From about 0.0001 to 0.1 weight % cellulase can be used.

Suitable cellulases are disclosed in U.S. Patent 4,435,307, Barbesgaard et al., issued March 6, 1984, incorporated herein by reference, which discloses fungal cellulase produced from Humicola insolens. Suitable cellulases are also disclosed in GB-A-2.075.028, GB-A-2.095.275 and DE-OS-2.247.832.

Examples of such cellulases are cellulases produced by a strain of Humicola insolens (Humicola grisea var. thermoidea), particularly the Humicola strain DSM 1800, and cellulases produced by a fungus of Bacillus N or a cellulase 212-producing fungus belonging to the genus Aeromonas, and cellulase extracted from the hepatopancreas of a marine mollusc (Dolabella Auricula Solander).

Any amylase suitable for use in a liquid detergent composition can be used in these compositions. Amylases include, for example, amylases obtained from a special strain of B.licheniformis, described in more detail in British Patent Specification No. 1,296,839 (Novo). Amylolytic proteins include, for example, Rapidase^R, International Bio-Synthetics, Inc. and Termamyl^R Novo Industries.

From about 0.0001% to 0.55, preferably 0.0005 to 0.1, wt. % amylase can be used.

Optional Ingredients - Detergent builders can optionally be included in the compositions herein. From 0 to about 50 weight % detergency builder can be used herein. Inorganic as well as organic builders can be used. When present, the compositions will typically comprise at least about 1% builder. Liquid formulations preferably comprise from about 3% to 30%, more preferably about 5 to 20%, by weight, of detergent builder.

Inorganic detergent builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates (exemplified by the tripolyphosphates, pyrophosphates, and glassy polymeric meta-phosphates), phosphonates, phytic acid, silicates, carbonates (including bicarbonates and sesquicarbonates), sulphates, and aluminosilicates. Borate builders, as well as builders containing borate-forming materials that can produce borate under detergent storage or wash conditions (hereinafter, collectively "borate builders"), can also be used. Preferably, non-borate builders are used in the compositions of the invention intended for use at wash conditions less than about 50°C, especially less than about 40°C.

Examples of silicate builders are the alkali metal silicates, particularly those having a SiO_2:Na_2O ratio in the range 1.6:1 to 3.2:1 and layered silicates, such as the layered sodium silicates described in U.S. Patent 4,664,839, issued May 12, 1987 to H. P. Rieck, incorporated herein by reference. However, other silicates may also be useful such as for example magnesium silicate, which can serve as a crispening agent in granular formulations, as a stabilizing agent for oxygen bleaches, and as a component of suds control systems.

Examples of carbonate builders are the alkaline earth and alkali metal carbonates, including sodium carbonate and sesquicarbonate and mixtures thereof with ultra-fine calcium carbonate as disclosed in German Patent Application No. 2,321,001 published on November 15, 1973, the disclosure of which is incorporated herein by reference.

Aluminosilicate builders are useful in the present invention. Aluminosilicate builders are of great importance in most currently marketed heavy duty granular

detergent compositions, and can also be a significant builder ingredient in liquid detergent formulations. Aluminosilicate builders include those having the empirical formula:

$$M_z(zAlO_2 \cdot ySiO_2)$$

wherein M is sodium, potassium, ammonium or substituted ammonium, z is from about 0.5 to about 2; and y is 1; this material having a magnesium ion exchange capacity of at least about 50 milligram equivalents of $CaCO_3$ hardness per gram of anhydrous aluminosilicate. Preferred alumino-silicates are zeolite builders which have the formula:

$$Na_z[(AlO_2)_z (SiO_2)_y] \cdot xH_2O$$

wherein z and y are integers of at least 6, the molar ratio of z to y is in the range from 1.0 to about 0.5, and x is an integer from about 15 to about 264.

Useful aluminosilicate ion exchange materials are commercially available. These aluminosilicates can be crystalline or amorphous in structure and can be naturally-occurring aluminosilicates or synthetically derived. A method for producing aluminosilicate ion exchange materials is disclosed in U.S. Patent 3,985,669, Krummel, et al., issued October 12, 1976, incorporated herein by reference. Preferred synthetic crystalline aluminosilicate ion exchange materials useful herein are available under the designations Zeolite A, Zeolite P (B), and Zeolite X. In an especially preferred embodiment, the crystalline aluminosilicate ion exchange material has the formula:

$$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}] \cdot xH_2O$$

wherein x is from about 20 to about 30, especially about 27. This material is known as Zeolite A. Preferably, the aluminosilicate has a particle size of about 0.1-10 microns in diameter.

Specific examples of polyphosphates are the alkali metal tripolyphosphates, sodium, potassium and ammonium pyrophosphate, sodium and potassium and ammonium pyrophosphate, sodium and potassium orthophosphate, sodium polymeta phosphate in which the degree of polymerization ranges from about 6 to about 21, and salts of phytic acid.

Examples of phosphonate builder salts are the water-soluble salts of ethane 1-hydroxy-1, 1-diphosphonate particularly the sodium and potassium salts, the water-soluble salts of methylene diphosphonic acid e.g. the trisodium and tripotassium salts and the water-soluble salts of substituted methylene diphosphonic acids, such as the trisodium and tripotassium ethylidene, isopyropylidene benzylmethylidene and halo methylidene phosphonates. Phosphonate builder salts of the aforementioned types are disclosed in U.S. Patent Nos. 3,159,581 and 3,213,030

issued December 1, 1964 and October 19, 1965, to Diehl; U.S. Patent No. 3,422,021 issued January 14, 1969, to Roy; and U.S. Patent Nos. 3,400,148 and 3,422,137 issued September 3, 1968, and January 14, 1969 to Quimby, said disclosures being incorporated herein by reference.

Organic detergent builders preferred for the purposes of the present invention include a wide variety of polycarboxylate compounds. As used herein, "polycarboxylate" refers to compounds having a plurality of carboxylate groups, preferably at least 3 carboxylates.

Polycarboxylate builder can generally be added to the composition in acid form, but can also be added in the form of a neutralized salt. When utilized in salt form, alkali metals, such as sodium, potassium, and lithium, or alkanolammonium salts are preferred.

Included among the polycarboxylate builders are a variety of categories of useful materials. One important category of polycarboxylate builders encompasses the ether polycarboxylates. A number of ether polycarboxylates have been disclosed for use as detergent builders. Examples of useful ether polycarboxylates include oxydisuccinate, as disclosed in Berg, U.S. Patent 3,128,287, issued April 7, 1964, and Lamberti et al., U.S. Patent 3,635,830, issued January 18, 1972, both of which are incorporated herein by reference.

A specific type of ether polycarboxylates useful as builders in the present invention also include those having the general formula:

CH(A)(COOX)-CH(COOX)-O-CH(COOX)-CH(COOX)(B)

wherein A is H or OH; B is H or -O-CH(COOX)-CH_2(COOX); and X is H or a salt-forming cation. For example, if in the above general formula A and B are both H, then the compound is oxydissuccinic acid and its water-soluble salts. If A is OH and B is H, then the compound is tartrate monosuccinic acid (TMS) and its water-soluble salts. If A is H and B is -O-CH(COOX)-CH_2(COOX), then the compound is tartrate disuccinic acid (TDS) and its water-soluble salts. Mixtures of these builders are especially preferred for use herein. Particularly preferred are mixtures of TMS and TDS in a weight ratio of TMS to TDS of from about 97:3 to about 20:80. These builders are disclosed in U.S. Patent 4,663,071, issued to Bush et al., on May 5, 1987.

Suitable ether polycarboxylates also include cyclic compounds, particularly alicyclic compounds, such as those described in U.S. Patents 3,923,679; 3,835,163; 4,158,635; 4,120,874 and 4,102,903, all of which are incorporated herein by reference.

Other useful detergency builders include the ether hydroxypolycarboxylates represented by the structure:

$$HO-[C(R)(COOM)-C(R)(COOM)-O]_n-H$$

wherein M is hydrogen or a cation wherein the resultant salt is water-soluble, preferably an alkali metal, ammonium or substituted ammonium cation, n is from about 2 to about 15 (preferably n is from about 2 to about 10, more preferably n averages from about 2 to about 4) and each R is the same or different and selected from hydrogen, C_{1-4} alkyl or C_{1-4} substituted alkyl (preferably R is hydrogen).

Still other ether polycarboxylates include copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid.

Organic polycarboxylate builders also include the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids. Examples include the sodium, potassium, lithium, ammonium and substituted ammonium salts of ethylenediamine tetraacetic acid, and nitrilotriacetic acid.

Also included are polycarboxylates such as mellitic acid, succinic acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, and carboxymethyloxysuccinic acid, and soluble salts thereof.

Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders of particular importance for heavy duty liquid detergent formulations, but can also be used in granular compositions.

Other carboxylate builders include the carboxylated carbohydrates disclosed in U.S. Patent 3,723,322, Diehl, issued March 28, 1973, incorporated herein by reference.

Also suitable in the detergent compositions of the present invention are the 3,3-dicarboxy-4-oxa-1,6-hexanedioates and the related compounds disclosed in U.S. Patent 4,566,984, Bush, issued January 28, 1986, incorporated herein by reference. Useful succinic acid builders include the C_5-C_{20} alkyl succinic acids and salts thereof. A particularly preferred compound of this type is dodecenylsuccinic acid. Alkyl succinic acids typically are of the general formula $R-CH(COOH)CH_2(COOH)$ i.e., derivatives of succinic acid, wherein R is hydrocarbon, e.g., C_{10}-C_{20} alkyl or alkenyl, preferably C_{12}-C_{16} or wherein R may be substituted with hydroxyl, sulfo, sulfoxy or sulfone substituents, all as described in the above-mentioned patents.

The succinate builders are preferably used in the form of their water-soluble salts, including the sodium, potassium, ammonium and alkanolammonium salts.

Specific examples of succinate builders include: laurylsuccinate, myristylsuccinate, palmitylsuccinate, 2-dodecenylsuccinate (preferred), 2-pentadecenylsuccinate, and the like. Laurylsuccinates are the preferred builders of this group, and are described in European Patent Application 86200690.5/0,200,263, published November 5, 1986.

Examples of useful builders also include sodium and potassium carboxymethyloxymalonate, carboxymethyloxysuccinate, cis-cyclo-hexane-hexacarboxylate, cis-cyclopentane-tetracarboxylate, water-soluble polyacrylates (these polyacrylates having molecular weights to above about 2,000 can also be effectively utilized as dispersants), and the copolymers of maleic anhydride with vinyl methyl ether or ethylene.

Other suitable polycarboxylates are the polyacetal carboxylates disclosed in U.S. Patent 4,144,226, Crutchfield et al., issued March 13, 1979, incorporated herein by reference. These polyacetal carboxylates can be prepared by bringing together, under polymerization conditions, an ester of glyoxylic acid and a polymerization initiator. The resulting polyacetal carboxylate ester is then attached to chemically stable end groups to stabilize the polyacetal carboxylate against rapid depolymerization in alkaline solution, converted to the corresponding salt, and added to a surfactant.

Polycarboxylate builders are also disclosed in U.S. Patent 3,308,067, Diehl, issued March 7, 1967, incorporated herein by reference. Such materials include the water-soluble salts of homo- and copolymers of aliphatic carboxylic acids such as maleic acid, itaconic acid and methylenemalonic acid.

Other organic builders known in the art can also be used. For example, monocarboxylic acids, and soluble salts thereof, having long chain hydrocarbyls can be utilized. These would include materials generally referred to as "soaps." Chain lengths of C_{10}-C_{20} are typically utilized. The hydrocarbyls can be saturated or unsaturated.

Other optional ingredients include soil release agents, chelating agents, clay soil removal/anti redeposition agents, polymeric dispersing agents, bleaches, brighteners, suds suppresors, solvents and aesthetic agents.

The detergent composition herein can be formulated as a variety of compositions, for instance as laundry detergents as well as hard surface cleaners or dishwashing compositions.

The compositions according to the present invention are further illustrated by the following examples.

EXAMPLE I

The following compositions are made by combining the listed ingredients in the listed proportions. In this example, one or more of the following peptide aldehydes are used:

Peptide aldehyde 1: CH_3O-(O)C-Phe-Gly-Ala-LeuH

Peptide aldehyde 2: CH_3N-(O)C-Phe-Gly-Ala-LeuH

Peptide aldehyde 3: CH_3O-(O)C-Phe-Gly-Ala-PheH

Peptide aldehyde 4: CH_3N-(O)C-Phe-Gly-Ala-PheH

Peptide aldehyde 5: CH_3SO_2Phe-Gly-Ala-Leu-H

Peptide aldehyde 6: CH_3SO_2Val-Ala-Leu-H

Peptide aldehyde 7: $C_6H_5CH_2O(OH)(O)P$-Val-Ala-Leu-H

Peptide aldehyde 8: $CH_3CH_2SO_2$-Phe-Gly-Ala-Leu-H

Peptide aldehyde 9: $C_6H_5CH_2SO_2$-Val-Ala-Leu-H

Peptide aldehyde 10: $C_6H_5CH_2O(OH)(O)P$-Leu-Ala-Leu-H

Peptide aldehyde 11: $C_6H_5CH_2O(OH)(O)P$-Phe-Ala-Leu-H

Peptide aldehyde 12: $CH_3O(OH)(O)P$-Leu-Gly-Ala-Leu-H.

Compositions	A	B	C	D	E	F
Linear alkyl benzene sulfonic acid	8.5	15	6.5	10	12.5	4
Sodium C_{12-15} alkyl sulfate	1	2	1	2	0	0
C_{14-15} alkyl 2.5 times ethoxylated sulfate	10	5	10.5	0	11	9
C_{12} glucose amide	0	0	9	0	0	5
C_{12-15} alcohol 7 times ethoxylated	3	10	4	7	2.5	0
Fatty acid	2	5	5	4	2	2
Citric acid	6	7	4	6	4	5
C_{12-14} alkenyl substituted, succinic acid	0	6	0	5	0	6
Sodium hydroxide	2	6	2	4	1	1.5
Ethanol	2	1.5	2	4	2	1.5
Monoethanolamine	6	5	4	0	0	0
1,2-Propanediol	12	10	5	5	4	6
Amylase (143 KNU/g)	0	0	0.1	0	0	0.2

Lipolase® (100KLU/g commercial solution)	0.5	0.2	0.5	0.5	0.4	0
Protease B (34 g/L commerical solution)	0.9	0	0.5	0	1.2	0
Savinase® (commercial solution)	0	0.3	0	0.4	0.2	0.3
Carezyme®	0.5	1	0.8	0	0.2	0.8
Peptide aldehydes 1-12	0.009	0.005	0.001	0.0005	0.0013	0.1
Boric Acid	0.25	0.5	0.75	5	2	8
Calcium Ions ($CaCl_2$)	--	--	--	--	0.01	--
Water and minors	Balance to 100%					

WHAT IS CLAIMED IS:

1. A liquid detergent composition comprising:
   a) from 1 to 95%, by weight of composition, of a detersive surfactant;
   b) an active proteolytic enzyme;
   c) a boron composition comprising a source of boric acid and a polyol; and
   d) a peptide aldehyde having the formula:

$$Z\text{-}B\text{-}NH\text{-}CH(R)\text{-}C(O)H$$

wherein B is a peptide chain comprising from 1 to 5 amino acid moieties; Z is an N-capping moiety selected from the group consisting of phosphoramidate $[(R"O)_2(O)P\text{-}]$, sulfenamide $[(SR")_2\text{-}]$, sulfonamide $[(R"(O)_2S\text{-}]$, sulfonic acid $[SO_3H]$, phosphinamide $[(R")_2(O)P\text{-}]$, sulfamoyl derivative $[R"O(O)_2S\text{-}]$, thiourea $[(R")_2N(O)C\text{-}]$, thiocarbamate $[R"O(S)C\text{-}]$, phosphonate $[R"\text{-}P(O)OH]$, amidophosphate $[R"O(OH)(O)P\text{-}]$, carbamate $(R"O(O)C\text{-})$, and urea $(R"NH(O)C\text{-})$, wherein each R" is independently selected from the group consisting of straight or branched C_1-C_6 unsubstituted alkyl, phenyl, C_7-C_9 alkylaryl, and cycloalkyl moieties, wherein the cycloalkyl ring may span C_4-C_8 and may contain one or more heteroatoms selected from the group consisting of O,N,and S; and R is selected from the group consisting of straight or branched C_1 - C_6 unsubstituted alkyl, phenyl, and C_7 - C_9 alkylaryl moieties.

2. A liquid detergent composition according to Claim 1 wherein said R moieties are selected from the group consisting of methyl, iso-propyl, sec-butyl, iso-butyl, -C_6H_5, -CH_2-C_6H_5, and -CH_2CH_2-C_6H_5.

3. A liquid detergent composition according to Claim 2 wherein said B peptide chains are selected from the group consisting of peptide chains having the amino acid sequences according to the general formula:

$$Z\text{-}A^5\text{-}A^4\text{-}A^3\text{-}A^2\text{-}A^1\text{-}NH\text{-}CH(R)\text{-}C(O)H$$

such that the following amino acids, when present, are :

A^1 is selected from Ala, Gly;

A^2 , if present, is selected from Val, Ala, Gly, Ile;

A^3 , if present, is selected from Phe, Leu, Val, Ile;

A^4 , if present, is any amino acid;

A^5 , if present, is any amino acid.

4. A liquid detergent composition according to Claim 3 wherein the N-terminal end is protected by one of the N-capping moiety protecting groups selected from the group consisting of carbamates, ureas, sulfonamides, phosphonamides,thioureas, sulfenamides, sulfonic acids, phosphinamides, thiocarbamates, amidophosphates, and phosphonamides.

5. A liquid detergent composition according to Claim 4 wherein the N-terminal end of said protease inhibitor is protected by a methyl, ethyl or benzyl carbamate [CH_3O-(O)C-; CH_3CH_2O-(O)C-; or $C_6H_5CH_2O$-(O)C-], methyl, ethyl or benzyl urea [CH_3NH-(O)C-; CH_3CH_2NH-(O)C-; or $C_6H_5CH_2NH$-(O)C-], methyl, ethyl or benzyl sulfonamide [CH_3SO_2-; $CH_3CH_2SO_2$-; or $C_6H_5CH_2SO_2$-], and methyl, ethyl or benzyl amidophosphate [CH_3O(OH)(O)P-; CH_3CH_2O(OH)(O)P-; or $C_6H_5CH_2O$(OH)(O)P-] groups.

6. A liquid detergent composition according to Claim 1 wherein said peptide aldehyde is selected from the group consisting of: CH_3SO_2Phe-Gly-Ala-Leu-H, CH_3SO_2Val-Ala-Leu-H, $C_6H_5CH_2O$(OH)(O)P-Val-Ala-Leu-H, $CH_3CH_2SO_2$-Phe-Gly-Ala-Leu-H, $C_6H_5CH_2SO_2$-Val-Ala-Leu-H, $C_6H_5CH_2O$(OH)(O)P-Leu-Ala-Leu-H, $C_6H_5CH_2O$(OH)(O)P-Phe-Ala-Leu-H, and CH_3O(OH)(O)P-Leu-Gly-Ala-Leu-H.

7. A liquid detergent composition according to Claim 1 wherein the boron composition comprises boric acid or a source of boric acid selected from the group consisting of boric oxide, borax, other alkali metal borates, substituted boric acids, and mixtures thereof; and the polyol is a diol.

8. A liquid detergent composition according to Claim 1 further comprising an effective amount of a source of calcium ions.

9. A liquid detergent composition according to Claim 8 comprising:
   a) from 8 to 70% of said detersive surfactant;
   b) from 0.001% to 5% of an active proteolytic enzyme;
   c) said boron composition comprising from 0.25% to 10%, by weight, of boric acid or a compound capable of forming boric acid in the composition and from 1% to 15%, by weight of the composition, of polyol;
   d) from 0.00001% to 5% of said peptide aldehyde; and
   e) from 0.01% to 1% of calcium ion.

10. A liquid detergent composition according to Claim 1 further comprising an effective amount one or more of the following enzymes: lipase, amylase, cellulase, and mixtures thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16626

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C11D C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP 0 583 534 A (PROCTER & GAMBLE) 23 February 1994 cited in the application see the whole document ---	1-10
Y	EP 0 381 262 A (UNILEVER NV ET AL.) 8 August 1990 cited in the application see abstract see page 2, line 47 - line 55 ---	1-10
Y	WO 92 03529 A (NOVONORDISK AS) 5 March 1992 see page 5, line 6 - line 12 see claims --- -/--	1,7,10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
21 January 1998	03.02.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pelli Wablat, B

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	US 4 537 706 A (SEVERSON JR ROLAND G) 27 August 1985 cited in the application see column 6, line 65 see column 7, line 60 - line 68 see column 8, paragraph 2 see claims ---	1,7-10
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